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LABORATORY INVESTIGATION

Change in energy reserves in different segments of the nephron during brief ischemia

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Change in energy reserves in different segments of the nephron during brief ischemia. Rat kidneys were made ischemic for 5 to 120 seconds. Segments of individual nephrons were dissected from freeze dried sections and analyzed for ATP, phosphocreatine, glycogen, glucose, glucose-6-phosphate, lactate and creatine kinase. ATP fell most rapidly in proximal convoluted and straight tubules (PCT, PST) and distal convoluted tubules (DCT), and most slowly in glomerulus and papilla. Phosphocreatine levels ranged fivefold and was highest in DCT, where it approached that of brain. Creatine kinase ranged 100-fold with lowest level in PCT, where the ischemic fall in phosphocreatine was so slow as to suggest a function other than that of an energy reserve. Glycogen varied tenfold from modest levels in distal segments to very low levels in PST, and was not used rapidly in any segment. Glucose consumption and lactate production were most rapid in distal portions. High–energy phosphate consumption for the first 7.5 seconds of ischemia, calculated from these data, indicates roughly–equal energy metabolism in proximal and distal segments, with lower levels in papilla, and especially in glomerulus. The absolute values suggest that the in vivo metabolic rate of the nephron continued almost unabated for 5 or 10 seconds of ischemia.

The kidney nephron consists of a series of organs with striking differences in structure, function and enzymatic composition. Less is known about differences in energy metabolism, although it is inferred from their enzyme content [1, 2] that proximal segments have a high oxidative metabolism and distal segments, a relatively–high glycolytic metabolism. This inference is supported by in vitro studies of kidney slices from regions enriched in proximal or distal tubules [3]. It has also recently been demonstrated by Bagnasco et al [4] that in vitro lactate production of the distal tubule and collecting ducts far exceeds that of the proximal tubule.

Energy metabolism in the kidney is of particular interest because of its exceptionally high value. The kidney shares with brain the highest metabolic rate in the body, (with the exception of maximally working muscle). Probably because of its high metabolic rate, the kidney is particularly sensitive to anoxia and ischemia. Moreover, some parts of the nephron are more sensitive to these insults than others. For example, the medullary thick ascending limb of Henle is particularly susceptible to damage from anoxia if filtration continues [5]. Paradoxically, during ischemia it is the S3 segment of the proximal tubule that is particularly vulnerable [6]. It should therefore be useful to have information about metabolite changes and energy expenditure in specific segments of the nephron in both of these conditions.

This study deals with one of them. It presents the results of analyses of six regions of the nephron and the papilla from kidneys subjected to 5 seconds to 120 seconds of complete ischemia. Identified segments of individual nephrons were dissected from freeze dried sections of fast frozen kidneys for analysis. The changes observed in ATP, phosphocreatine, glucose, glycogen and lactate permit assessment of segmental differences in in situ glycolytic and glycogenolytic capacity and in demand for high energy phosphate.

Methods

Animals

Male Sprague–Dawley rats weighing 300 to 350 g were used. They were given trypan blue (0.1 mg/g) intraperitoneally 18 to 24 hours before the experiment. This dye specifically stains proximal convoluted tubules and aids visualization for dissection [7].

Kidney samples

Brief light ether anesthesia was used, usually three minutes overall. Kidneys were plunged into Freon-12 (CCl_2F_2) (chilled in liquid N2 to its freezing point) within one second after removal or after incubation under mineral oil at 37°C for 5 to 120 seconds. The frozen kidneys were stored at −80°C until prepared for analysis. Blocks of tissue were mounted and sectioned at 16 μm without allowing the temperature to rise above −20°C. The sections were freeze dried at −35°C under vacuum and stored at −80°C under vacuum.

The general technique for isolation and weighing small identified samples has been described in detail and the application to microtome sections reported [8]. Various parts of the nephron were identified as described before [9] by characteristic size, color, and texture. Samples consisted of either a central slice of a glomerulus, a 75 to 150 μm length of tubule, or a patch from the papilla below the inner stripe of the medulla. Proximal segments were from the early part of the convoluted tubule (PCT, deeply stained with trypan blue) or early or late portions

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Table 1. Flow chart for assays

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7	Standards[f] μM
ATP	0.1 μl 0.1 M NaOH[a]	0.2 μl SpR RT 20 min	0.2 μl 0.15 M NaOH[a]	5 μl TCR RT 2 hr 30,000X	1 μl 1 M NaOH[a]	5 μl into 1 ml 6PG Rgt		0.5–2
Phospho-creatine	0.05 μl 0.02 M NaOH[a]	0.05 μl[c] ATP Rgt RT 20 min	0.2 μl SpR RT 30 min	0.2 μl 0.2 M NaOH[a]	5 μl TCR RT 15 hr 120,000X	1 μl 1 M NaOH[a]	5 μl into 1 ml 6PG Rgt	0.5–2
Glycogen	0.2 μl 0.02 M NaOH[a]	0.2 μl SpR RT 30 min	0.2 μl Glc Rgt RT 15 min	0.2 μl 0.2 M NaOH[a]	7.5 μl TCR 38° 2 hr 60,000X	2 μl 1 M NaOH[a]	7 μl into 1 ml 6PG Rgt	0.1–1
Glucose	0.1 μl 0.02 M HCl[b]	0.3 μl SpR RT 20 min	0.2 μl 0.12 M NaOH[a]	5 μl TCR 38° 1 hr 15,000X	1 μl 0.5 M NaOH[a]	5 μl into 1 ml 6PG Rgt		1–5
Glucose-6-phosphate	0.05 μl 0.02 M HCl[b]	0.1 μl SpR RT 30 min	0.1 μl 0.1 M NaOH[a]	1 μl TCR RT 20 hr 200,000X	10 μl 0.05 M NaOH[a]	10 μl into 1 ml 6PG Rgt		0.05–0.2
Lactate	0.05 μl 0.02 M NaOH[a]	0.1 μl SpR RT 30 min	3 μl 0.05 M NaOH[a]	3 μl into 50 μl DCR 25° 60 min[e]	95° 3 min	1 ml mal Rgt		2–10
Creatine-kinase	5 μl SpR 20°, 60 min	5 μl 0.12 M NaOH[a]	5 μl into 50 μl 50 μl TCR 1 hr[d]	95° 3 min	1 ml 6PG Rgt			50–150 (ATP)

Abbreviations are: Rgt, reagent; SpR, specific reagent; ATP Rgt, ATP removing Rgt; Glc Rgt, glucose Rgt; TCR, NADP cycling reagent; DCR, NAD cycling reagent; 6PG Rgt, 6-phosphogluconate measuring Rgt; Mal Rgt, malate measuring reagent.

[a] Heated 20 min at 80°C

[b] Heated 20 min at 60°C

[c] 0.05 μl of 0.15 M HCl added and left 10 min or more at room temperature to destroy NADPH before proceeding

[d] 3000X for PCT and PST, 400X for other structures

[e] 5000X

[f] Concentrations in the first reagent

of the straight tubule (PST_E or PST_L). Distal segments were from the medullary thick ascending limb of Henle (TAL) or the convoluted segment (DCT). The samples (5 to 40 ng dry wt) were weighed ± 2% on a quartz fiber balance [8]. The value for each structure of each kidney is based on three to 10 replicate assays.

Analytical methods

Enzymes were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA) or Boehringer–Mannheim (Ridgefield, Connecticut, USA) except beef heart lactic dehydrogenase which was from Cooper Biomedical. Most of the other biochemicals were from Sigma. All reagents were of analytical grade.

Obviously the amounts of the various metabolites per sample were very small. Control levels ranged from 2×10^{-15} mol for glucose-6-phosphate to 10^{-12} mol for glucose. Nevertheless, except in the case of lactate, no special precautions were required other than those demanded for analytical precision at any level. Reagents of analytical grade did not require special treatment, except in the few instances noted. This is largely due to the analytical amplification by enzymatic cycling which is used in each assay. This reduces trace impurities to insignificance, relative to the substance specifically measured.

The methods were based on an enzyme reaction or sequence of reactions which result in oxidation or reduction of a pyridine nucleotide. Sensitivity is then highly amplified by using the pyridine nucleotide product to catalyze a two–step enzyme reaction ["enzymatic cycling", 8]. Improved cycling methods were used for NAD [10] and NADP [11]. These papers may be consulted for details including composition of the "indicator reagents", that is, the 6-phosphogluconate and malate measuring reagents of Table 1.

In general the assays were patterned after those developed for single muscle fibers [12]. A major difference is that extraction into $HClO_4$ was omitted. The weighed sample was loaded through oil into a small droplet of NaOH or HCl in one of 60 shallow wells drilled in a Teflon block [8] (DuPont, Wilmington, Delaware, USA). The loaded block was heated to destroy tissue enzymes. The necessary reagents were then added in sequence as required, with final transfer from the oil well to a fluorometer tube for last step(s) and ultimate fluorescence reading.

Table 1 is a flow chart for the assays. The composition of specific reagents and explanatory notes are as follows.

ATP

The specific reagent contained 140 mM Tris-HCl buffer, pH 8.1, 300 μM glucose, 2 mM $MgCl_2$, 75 μM $NADP^+$, 0.06% bovine serum albumin (BSA), 0.4 mM dithiothreitol, 10 μg/ml yeast hexokinase (EC 2.7.1.1.) and 5 μg/ml of 30% pure yeast glucose-6-phosphate dehydrogenase (EC 1.1.1.49).

Phosphocreatine

Tissue ATP must be removed before measuring phosphocreatine, since ATP formation is part of the analytical reaction sequence. This removal was done with an "ATP rgt" (Table 1), similar to the specific reagent of the ATP assay. Its composition was 70 mM Tris-HCl buffer, pH 8.1, 350 μM glucose, 2 mM $MgCl_2$, 80 μM $NADP^+$, 0.08% BSA, 10 μg/ml hexokinase, 4 μg/ml glucose-6-phosphate dehydrogenase and 300 μM ADP. The ADP is included since it is required for the specific enzyme step, and this pretreatment provides a convenient way to remove contaminating ATP, which is nearly always present in commercial ADP.

The specific reagent for phosphocreatine contained 125 mM imidazole-HCl buffer, pH 7.0, 90 μM glucose, 3 mM $MgCl_2$, 80 μM $NADP^+$, 0.05% BSA, 0.2 mM dithiothreitol, 2 mM EDTA, 200 μg/ml muscle creatine kinase (EC 2.9.3.2), 12 μg/ml hexokinase, and 8 μ/ml glucose-6-phosphate dehydrogenase.

Glycogen

The specific reagent hydrolyzes glycogen to glucose at an acid pH. A second reagent (Glc Rgt) then converts glucose at pH 8 to 6-phosphogluconate, forming NADPH. This is a modification of a procedure [13] based on the method of Lust, Passonneau and Lowry [14].

The specific reagent contained 100 mM sodium acetate buffer, pH 4.6 (base:acid, 1:1), 0.04% BSA and 4 μg/ml of amylo-1,4-glucosidase (EC 3.2.1.3) from *Aspergillus niger*. The Glc Rgt contained 100 mM Tris-HCl buffer, pH 8.7 (base:acid, 4:1), 1 mM ATP, 100 μM $NADP^+$, 6 mM $MgCl_2$, 0.07% BSA, 1 mM dithiothreitol, 1.5 mM EDTA, 8 μg/ml hexokinase, and 2 μg/ml glucose-6-phosphate dehydrogenase.

The mixture of the acetate and Tris buffers yields a pH of about 8. For distal structures, which contain relatively high glycogen, the reagent volumes for steps 2–4 were increased fivefold (to 1 μl), and the cycling amplification was reduced from 60,000 to 20,000-fold.

Glucose

The specific reagent contained 75 mM Tris-HCl buffer, pH 8.1, 140 μM ATP, 24 μM $NADP^+$, 2 mM $MgCl_2$, 0.06% BSA, 0.4 mM dithiothreitol, 8 μg/ml hexokinase, and 4 μg/ml glucose-6-phosphate dehydrogenase.

Glucose-6-phosphate

The specific reagent contained 75 mM Tris-HCl, pH 8.1, 15 μM $NADP^+$, 0.07% BSA, 0.4 mM dithiothreitol, and 6 μg/ml glucose-6-phosphate dehydrogenase. Note that because of the very low levels of this metabolite, particularly during ischemia, very high amplification was required (200,000-fold), which necessitated a 20 hour incubation at the cycling step (Table 1).

Lactate

The specific reagent contained 100 mM 2-amino-2-methyl-1-propanol buffer, pH 9.8, 0.5 mM NAD^+, 0.5 mM monosodium glutamate, 0.05% BSA, 50 μg/ml beef heart lactate dehydrogenase (EC 1.1.1.28) and 100 μg/ml alanine transaminase (EC 2.6.1.2). The glutamate and transaminase were added to pull the reaction to completion by removing the pyruvate formed.

Lactate contamination can be very troublesome because of the extremely high levels on the hands. All glassware was carefully rinsed just before use and gloves worn in making up the reagents and in handling reaction vessels.

Creatine kinase

The method is adapted from an earlier procedure used for assay of single muscle fibers [15], primarily to introduce an enzymatic cycling step.

The specific reagent contained 100 mM imidazole acetate buffer, pH 6.8, 10 mM phosphocreatine, 1 mM ADP, 3 mM glucose, 200 μM $NADP^+$, 15 mM magnesium acetate, 10 mM AMP, 20 μM P^1, P^5-di(adenosine-5^1)pentaphosphate, 0.5 mM EDTA, 5 mM dithiothreitol, 0.02% BSA, 15 μg/ml hexokinase, and 1 μg/ml glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*.

The reagent is almost identical to that used before, except for the addition of the diadenosine pentaphosphate, which is a potent inhibitor of adenylate kinase [16]. The AMP, which had been included in the original reagent for the same purpose, was retained. The procedure has been altered to measure the much lower activities in the nephron by introducing the enzymatic cycling amplification steps.

There was found to be a small "tissue blank", that is, a measurable increase in the final reading if tissue was incubated with reagent lacking phosphocreatine. This was therefore measured for each structure and the results corrected accordingly, although the difference was insignificant except in the case of the proximal tubule segments, where the blank was substantially larger than the contribution from creatinine kinase itself.

Results

ATP, phosphocreatine and creatine kinase

Normal values. As has been shown before [17], ATP levels vary about twofold along the nephron from about 10 mmol kg^{-1} (dry wt) in the glomerulus, proximal tubule segments, and papilla to 16 to 17 mmol kg^{-1} in distal tubule segments (Figs. 1, 2, Table 2).

Phosphocreatine. Phosphocreatine is more variable than ATP, ranging fivefold from about 2 mmol kg^{-1} in the proximal straight tubule (PST), thick ascending limb of Henle (TAL) and papilla to 10 mmol kg^{-1} in the distal convoluted tubule (DCT) (Figs. 1, 3, Table 2). In contrast to ATP, phosphocreatine is about three times higher in the glomerulus and proximal convoluted tubule (PCT) than in the PST, and only a fifth as high in the TAL as in the DCT.

Creatine kinase. Creatine kinase was also measured in the same nephron structures (Fig. 1), because of major differences in the rate of ischemic change in phosphocreatine among the different structures. Surprisingly, the activity of this enzyme was found to vary 100-fold from a high in DCT to a low in PCT. Moreover, activities were completely unrelated to phosphocreatinine levels, except that one of the highest levels was in the DCT where phosphocreatine concentration also reached its peak. Nevertheless, this peak creatine kinase level is only about 5% of that in rat skeletal muscle and 20% of that in brain.

Changes with ischemia. ATP, on a percentage basis, fell most rapidly in the proximal tubule segments, dropping about 50% in the first five seonds, and leveling off after 30 seconds at about

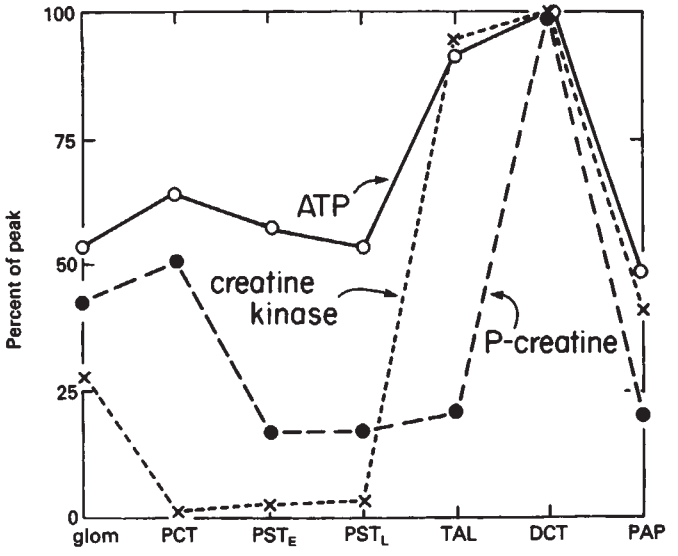

Fig. 1. *Distribution of ATP (○——○), phosphocreatine (●-----●), and creatine kinase (×----×) along the nephron and in the papilla as percentages of peak values.* The peak levels were 17.4 and 10.1 mmole · kg^{-1} (dry wt) for ATP and phosphocreatine, respectively, and 18.0 ± 1.2 (SE) mole · kg^{-1} (dry wt) h^{-1} at 20°C for creatine kinase. The minimum activity for creatine kinase was 0.17 ± 0.12 mole · kg^{-1} hr^{-1} in PCT.

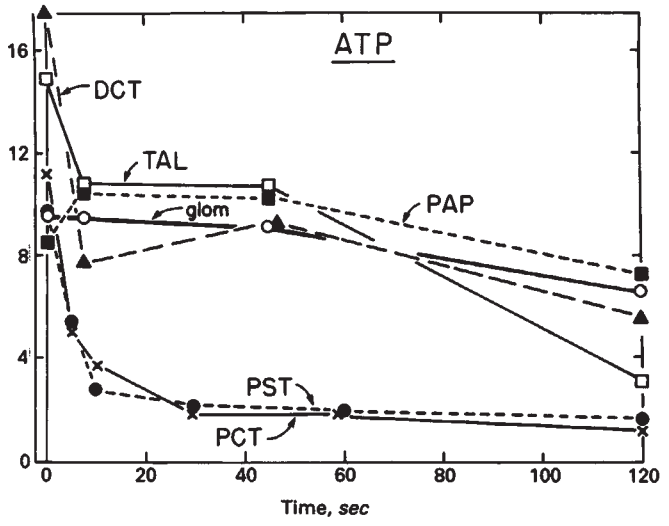

Fig. 2. *Effect of ischemia on ATP levels in 6 parts of the nephron and in the papilla.* Concentrations are mmol · kg^{-1} (dry wt). Except as indicated, each value is the average for 3 to 8 kidneys. The 7.5 and 45 sec values are the averages of data for 5 and 10 sec, and 30 and 60 sec periods of ischemia respectively. Symbols are: (▲) DCT, (□) TAL, (■) PAP, (○) glom, (●) PST, and (×) PCT.

20% of control (Fig. 2). The initial change was about as rapid in the DCT, but subsequent change was slow and the level at 45 seconds was still half of control. In TAL there was an initial 30% drop with no further change by 45 seconds, but with another drop to 20% of control by 120 seconds. The decreases in TAL at 7.5 seconds and 45 seconds were significantly smaller ($P < 0.05$) than for either PCT or PST. ATP underwent minimal changes in glomerulus and papilla; the levels at 120 seconds were still 70% and 85% of control, respectively.

Phosphocreatine fell more slowly than ATP in PCT and PST, and more rapidly than ATP in glomerulus and papilla (Fig. 2, Table 1). The rates were similar to each other in TAL and DCT. By 120 seconds the decrease in phosphocreatine, on a percentage basis, was as great or greater than that of ATP in all structures except the PCT. A greater change in phosphocreatine than in ATP would be anticipated on the basis of the equilibrium constant of the creatine kinase reaction. An explanation for the delay in phosphocreatine change in the proximal tubule may be the extremely low level of creatine kinase in that portion of the nephron.

Glycogen and glucose

(These metabolites are considered together since they both are potential sources of lactate and therefore high energy phosphate during ischemia.)

Control levels. Glycogen was very low in the proximal segments but higher by a factor of 10 in TAL and papilla (Fig. 4, Table 2). Limited data for DCT indicated a glycogen level in the same range as the TAL. The glomerulus contained glycogen at about half the level of the TAL. We are not aware of previous data on glycogen in nephron segments, but Needleman, Passonneau and Lowry [18] measured this metabolite in successive subdivisions of rat kidney from cortex to papilla and found levels that would be about as expected from the component nephron structures present in each subdivision. For example, glycogen concentrations in the outermost and innermost layers were about 5 and 18 mmol kg^{-1} (dry wt), respectively.

Glucose varied along the nephron much less than glycogen in control kidney, with highest levels in the glomerulus and DCT, and lowest in PST, TAL and PAP (Fig. 5, Table 2). The concentration in PCT was significantly higher than in the PST. This normal distribution agrees well with that reported earlier [19].

Changes in glycogen and glucose with ischemia. There were no significant changes in glycogen during the first 45 seconds of ischemia in any of the nephron portions, except for a drop in the PCT which was very small in absolute terms (Fig. 4, Table 1). By 120 seconds the levels had fallen at most by a third, but only the decreases in glomerulus, PCT and TAL were statistically significant ($P < 0.05$).

Glucose decreased significantly ($P < 0.05$) in all regions except PST$_L$ during 120 seconds of ischemia by amounts ranging from 24% in PST$_E$ to 93% in TAL (Fig. 5, Table 2). The average decrease for the three distal portions (TAL, DCT and papilla) was 69%, whereas in the proximal portions (glomerulus, PCT and PST) the decrease averaged only 27%. The most rapid change occurred in TAL: 40% in 7.5 seconds, the only structure showing a significant decrease at this time ($P < 0.05$). By 45 seconds, levels in the three distal portions had fallen an average of 43% ($P < 0.01$ for TAL and DCT), whereas in the proximal portions the average decrease was only 7%.

It should be useful to compare the contributions of glucosyl units to the metabolic pool from glycogen and glucose during ischemia. During the full 120 seconds the contribution from glucose exceeded that from glycogen in all regions. In the proximal structures the contribution from glycogen was especially small: 15% in the case of the glomerulus, 10% in PCT and

Table 2. Effect of ischemia on metabolite levels in different segments of the nephron

	Ischemia *sec*		Glomerulus	PCT	PST_E	PST_L	TAL	DCT	PAP
ATP	0	(5)	9.5 ± 0.5	11.2 ± 1.4	10.1 ± 1.2	9.4 ± 1.4	14.8 ± 1.4	17.4 ± 2.1	8.6 ± 0.4
	7.5	(8)	9.5 ± 0.9	4.38 ± 0.58	4.23 ± 0.85	4.02 ± 0.69	10.8 ± 0.4	7.8 ± 1.0	10.5 ± 0.7
	45	(6)	9.1 ± 1.0	1.93 ± 0.16	1.82 ± 0.14	2.45 ± 0.15	10.8 ± 1.8	9.3 ± 1.2	10.2 ± 1.2
	120	(3)	6.8 ± 0.3	1.24 ± 0.20	1.85 ± 0.14	1.65 ± 0.21	3.16 ± 0.39	5.7 ± 1.1[d]	7.2 ± 0.6
Phosphocreatine	0	(5)	4.37 ± 0.40	5.15 ± 0.20	1.67 ± 0.56	1.76 ± 0.69	2.18 ± 0.64	10.1 ± 0.3[d]	2.02 ± 0.58
	7.5	(7)	4.29 ± 0.37	6.11 ± 0.42	1.45 ± 0.24	1.47 ± 0.27	1.65 ± 0.16	7.3 ± 0.7	2.56 ± 0.38
	45	(6)	1.98 ± 0.32	4.59 ± 0.34	1.33 ± 0.22	1.08 ± 0.21	1.31 ± 0.18	3.95 ± 0.44	2.38 ± 0.43
	120	(3)	0.83 ± 0.05	2.76 ± 0.13	0.12 ± 0.02	0.07 ± 0.03	0.31 ± 0.01	2.59 ± 0.01[d]	0.80 ± 0.08
Glycogen	0	(5)	7.9 ± 0.4	2.48 ± 0.32	1.81 ± 0.34	1.83 ± 0.18	21.2 ± 0.4		18.7 ± 2.9
	7.5	(8)	9.3 ± 0.7	1.42 ± 0.24	1.50 ± 0.23	1.73 ± 0.26	24.6 ± 2.7		21.6 ± 2.5
	45	(8)	9.9 ± 1.2	1.56 ± 0.16	1.56 ± 0.13	1.30 ± 0.10	20.4 ± 3.9		21.1 ± 2.1
	120	(3)	5.2 ± 0.6	1.19 ± 0.09	1.76 ± 0.27	1.78 ± 0.60	14.2 ± 2.3		14.0 ± 1.4
Glucose	0	(5)	35.7 ± 2.9	31.8 ± 3.4	23.5 ± 2.0	20.6 ± 2.2	20.5 ± 3.0	41.2 ± 2.5	20.4 ± 3.0
	7.5	(7)	33.6 ± 1.1	29.1 ± 0.4	26.0 ± 1.3	22.9 ± 2.1	12.3 ± 2.5	33.6 ± 4.9	20.8 ± 0.8
	45	(7)	30.4 ± 2.0	29.8 ± 2.2	25.8 ± 1.7	20.1 ± 2.6	6.3 ± 2.1	28.6 ± 1.3	14.4 ± 2.1
	120	(3)	23.2 ± 1.1	21.9 ± 1.3	17.8 ± 0.4	16.7 ± 0.5	1.4 ± 0.3	18.6[c]	8.1 ± 2.0
Glucose-6-phosphate	0	(4)	0.589 ± 0.039	0.125 ± 0.018	0.314 ± 0.013	0.502 ± 0.060	0.304 ± 0.055		0.321 ± 0.040
	7.5	(7)	0.488[a]	0.133 ± 0.038	0.201 ± 0.047	0.281 ± 0.059	0.149 ± 0.009		0.481[a]
	45	(6)	0.390[b]	0.071 ± 0.006	0.113 ± 0.015	0.161 ± 0.017	0.164 ± 0.016		0.51 ± 0.06[d]
	120	(3)	0.193 ± 0.013	0.060 ± 0.012	0.054 ± 0.007	0.046 ± 0.006	0.124 ± 0.008		0.235 ± 0.049
Lactate	0	(5)	18.3 ± 1.1	15.6 ± 3.0	11.6 ± 1.6	10.1 ± 0.4	23.1 ± 2.0		22.5 ± 1.5
	7.5	(7)	23.7 ± 3.0	18.6 ± 2.4	16.6 ± 1.5	13.9 ± 1.3	29.9 ± 3.5		33.3 ± 3.5
	45	(7)	23.7 ± 3.0	18.7 ± 2.0	16.2 ± 1.6	17.0 ± 3.3	43.4 ± 4.6		41.5 ± 4.0
	120	(3)	29.4 ± 4.3	23.0 ± 1.3	22.4 ± 4.9	21.7 ± 3.3	53.6 ± 1.5		57.7 ± 0.9

The levels are all recorded as mmole kg^{-1} (dry wt.) ± S.E. for the number of kidneys in parenthesis unless noted. Three to ten samples (usually 5) of each nephron segment of each animal were analyzed. The figures at 7.5 sec consist of pooled data for 5 and 10 sec of ischemia. They have been combined because there were few significant differences between the two groups, and interpretation became easier with a larger number of kidneys represented. For the same reason, data for kidneys exposed to 30 and 60 sec of ischemia have been combined to give the 45 sec values.

[a] One kidney at 10 sec
[b] One kidney at 30 sec
[c] One kidney
[d] Two kidneys

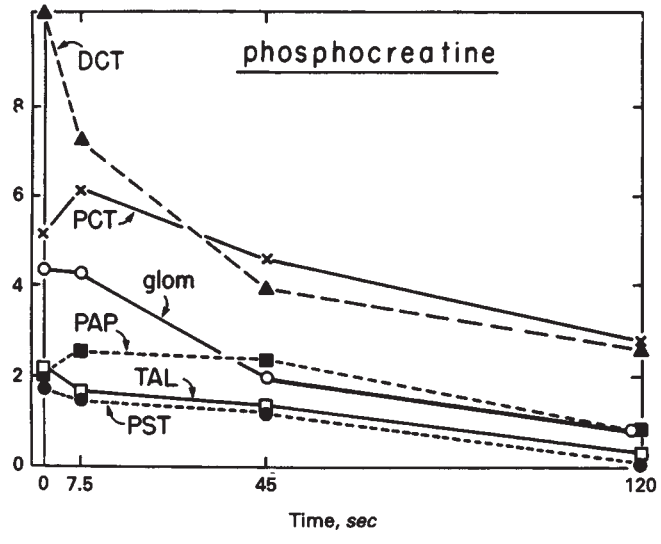

Fig. 3. *Effect of ischemia on phosphocreatine in 6 parts of the nephron and in the papilla.* Symbols are the same as in Fig. 2.

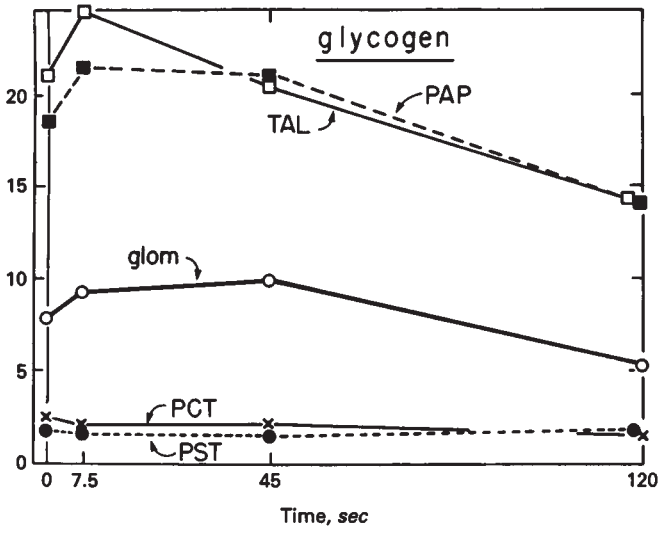

Fig. 4. *Effect of ischemia on glycogen levels in 5 parts of the nephron and in the papilla.* Concentrations are given as glycosyl units, mmol kg^{-1} (dry wt). Symbols are the same as in Fig. 2.

1% in PST. In contrast, glycogen contributed almost 30% of the total in TAL and papilla.

Glucose-6-phosphate and lactate

These two metabolites are considered together since the rate of change in lactate is a measure of the flux on the glycolytic pathway below glucose-6-phosphate, and changes in glucose-6-phosphate combined with a knowledge of the flux can be used to assess changes in degree of control being exercised at the phosphofructokinase step.

Control levels. As reported earlier [17], highest control glu-

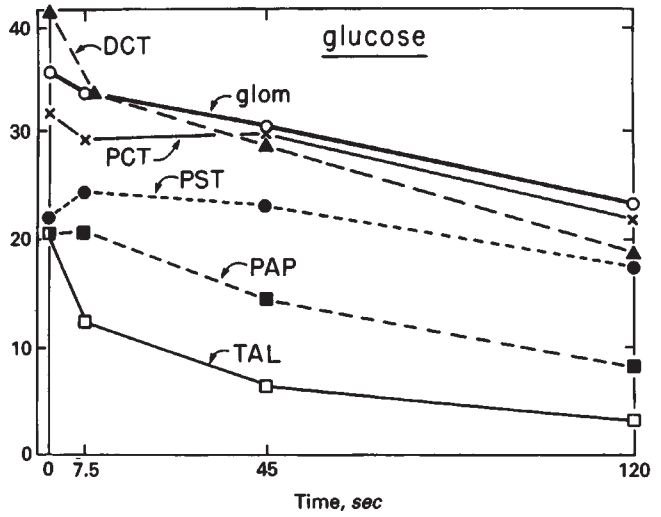

Fig. 5. *Effect of ischemia on glucose levels in 6 parts of the nephron and in the papilla.* Symbols are the same as in Fig. 2.

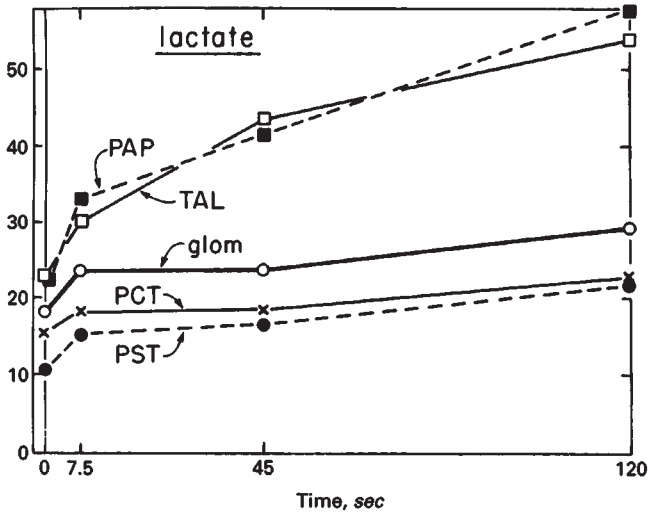

Fig. 7. *Effect of ischemia on lactate levels in 4 parts of the nephron and in the papilla.* Symbols are the same as in Fig. 2.

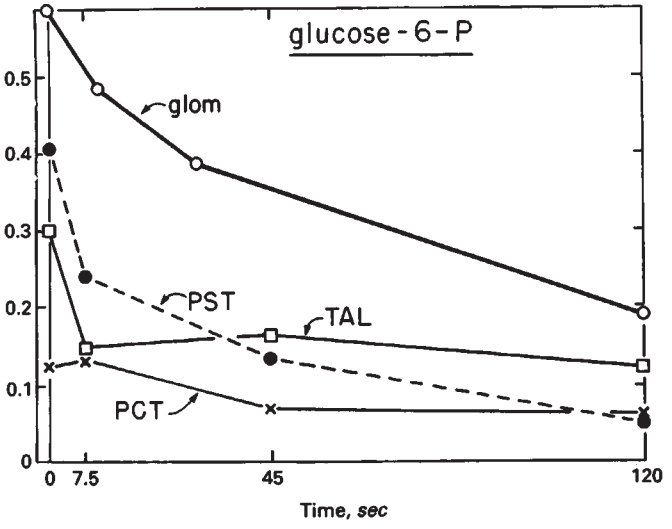

Fig. 6. *Effect of ischemia on glucose-6-phosphate levels in 4 parts of the nephron and in the papilla.* Symbols are the same as in Fig. 2.

cose-6-phosphate levels are in the glomerulus and the late portion of the PST (Fig. 6, Table 2). Lactate concentrations ranged twofold from lowest levels in the PST and highest in TAL and PAP (Fig. 7, Table 2).

Changes with ischemia. Glucose-6-phosphate decreased in all structures with ischemia but by different amounts and rates (Fig. 5, Table 2). The TAL and late PST (PST_L) levels fell most rapidly at first, and they continued to fall at a rapid rate in the PST_L but not in the TAL. The greatest change in 120 seconds occurred in the PST (−87%). Elsewhere the decreases ranged from 67% in the glomerulus to 27% in PAP.

Lactate increases with ischemia differed sharply in absolute terms between the proximal and distal structures, but less so percentage-wise (Fig. 7, Table 2). The increments in the glomerulus, PCT, and PST were only 7 to 12 mmol · kg^{-1}. The increments in TAL and papilla were much greater, 30 and 35 mmol · kg^{-1}, respectively.

The rise in lactate during ischemia should approximate the fall in glucose plus glycogen (two lactates per glucosyl unit). A test of this, with the 120 second data, showed good agreement in two cases: for PST 11.2 mmol · kg^{-1} lactate observed, 9.7 mmol · kg^{-1} calculated; for papilla 35 mmol · kg^{-1} lactate observed, 34 mmol · kg^{-1} calculated. However, in the other regions, lactate calculated was decidedly more than found: 70% too high in TAL and three times too high in PCT and glomerulus.

In both glomerulus and PCT, the discrepancies amount to only about 25% of the initial glucose content. It seems possible that when the blood supply is cut off and capillary pressure falls, enough capillary blood and glomerular fluid, both high in glucose, may leave the glomerulus to account for the discrepancy. Similarly, it is conceivable that in the PCT, which is the segment most active in glucose transport, this function may continue long enough to transfer a substantial amount of glucose into the interstitial space.

High energy phosphate consumption. During complete ischemia, that is, with oxidation blocked and no metabolite washout, it is possible to approximate the high energy phosphate (~P) consumption ("metabolic rate") from the decreases in ATP and phosphocreatine and the increase in lactate [20]. Figure 8 records this calculation for the rate during the first 7.5 seconds of ischemia. The assumption is made that lactate came exclusively from glucose, generating 1 mol of ~P per mole, and that (as calculated from whole kidney data reported in [21]) the dephosphorylated ATP went 80% to AMP and 20% to ADP, that is, 1.8 mole of ~P per mole of ATP decrease. These estimates probably err somewhat on the low side because no allowance has been made for a contribution from nucleotide triphosphates other than ATP, and any lactate generated from glycogen would yield 1.5 mol ~P per mole of glucosyl units.

The proximal tubule segments had about the same calculated ~P consumption rate as the TAL, but 67 to 80% came from ATP breakdown, whereas in the TAL only 50% came from ATP with nearly an equal proportion from glycolysis. It is probable that the highest ~P use was in the DCT, but reliable lactate data are not available. The lowest ~P use was in the glomerulus

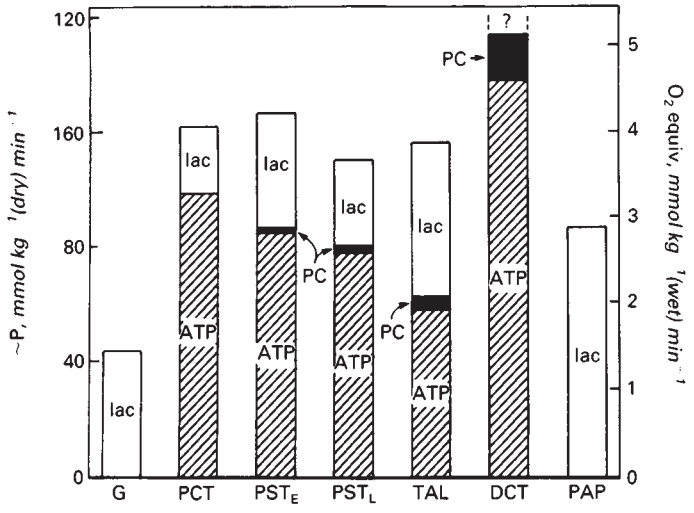

Fig. 8. *Calculated high energy phosphate consumption for the first 7.5 seconds of ischemia in different parts of the nephron and in papilla.* The assumptions on which the calculations are based are given in the text, including the conversion of ~P to equivalent O^2 consumption.

followed by the PAP, with no significant contribution during the first 7.5 seconds from ATP or phosphocreatine in either structure.

Figure 8 also shows the O_2 consumption equivalent to the ~P use. This is simply based on a P/O ratio of 3 and has been calculated on a wet weight basis, assuming a 1 to 5 ratio between dry and wet mass. Nishiitsutsuji-Uwo, Ross and Krebs [22] observed an O_2 consumption of about 5 mmol · kg^{-1} (wet) min^{-1} for perfused rat kidney. This agrees surprisingly well with the (probably slightly low) estimates of Figure 8 and suggests that the in vivo metabolic rate is maintained for a brief period after the blood supply is cut off.

Discussion

The present results with defined segments of the nephron are quite compatable with earlier data on the effects of ischemia on whole kidney and kidney cortex [21, 23], and on sequential thick tangential-slices of quick frozen kidneys [18]. Similarly, the relatively slow fall in ATP in papilla reported here confirms the finding of Jones and Welt [24]. The approximate agreement of calculated ~P consumption with that corresponding to oxygen consumption in vivo makes it likely that the data for the individual nephron portions reflects the in vivo metabolic rate. On this assumption, the metabolic rate in the glomerulus is only about a third that of the proximal tubule segments, and glycolysis was sufficient to maintain the ATP level for at least 45 seconds of ischemia, as was also true for the papilla.

The similar rates of ~P consumption in different tubule segments (with the probable exception of the DCT) in spite of differing sources of energy, agrees with earlier macro slice data [18]. In that study it was shown that slices from the outer and inner half of the kidney consumed during 30 seconds similar amounts of ~P, but the outer slices had used much more ATP and phosphocreatine, the inner slices had produced more lactate, giving a calculated ~P consumption that was similar in all slices.

Since brain and kidney have similar metabolic rates, it may be useful to compare the metabolic changes with ischemia in the two organs. In mouse brain [20] ATP fell only about 10% during the first 7.5 seconds of ischemia, as compared to a 60% drop in kidney PCT and PST. Nevertheless, the calculated ~P consumption was similar because in brain there was a 35% drop in phosphocreatine and a 65% increase in lactate, whereas in PCT and PST the contributions to ~P consumption were small from lactate formation and almost nil from phosphocreatine.

The ATP, creatine phosphate system. Creatine phosphate has received almost no attention in kidney, probably because the level in whole kidney is low compared to muscle and brain. Attempts to measure phosphocreatine in whole kidney in vivo with NMR have failed because of the difficulty in avoiding signals from the exceedingly high levels of phosphocreatine in adjacent muscle [25]. Measurements have been made with isolated perfused rat kidney. Phosphocreatine signals were observed at just above the limit of detection (equivalent to 1 to 2 mmol per kg dry weight) [25, 26]. This is not far below what might be expected from the present in vivo data, considering the fact that segments with high levels are diluted with segments with very low levels and the fact that NMR only detects "free phosphocreatine." Low levels in many tissues have been dismissed as probably due to the presence of vascular smooth muscle. Gerlach, Bader, and Schwoerer [21] found a level of about 0.4 mmol · kg^{-1} wet weight (2 mmol · kg^{-1} dry wt in rat kidney) and attributed this to blood vessel smooth-muscle. This could be the source of the phosphocreatine in the glomeruli and papilla, but cannot explain the levels in the cleanly-dissected tubule segments. The present finding of a high level in the DCT and a substantial level in the PCT increases interest in this high energy phosphate reserve. (The DCT level approaches that of normal brain.)

The equilibrium constant for the creatine kinase system strongly favors ATP formation. In keeping with this, it has long been known, from studies of brain and muscle, that phosphocreatine falls before ATP when high-energy phosphate generation fails to meet demand (ischemia, severe exercise, etc.). It therefore comes as a surprise that in the kidney segments, especially the PCT, ATP falls faster and further than phosphocreatine. A possible explanation is the relatively low level of creatine kinase compared to brain and muscle. The activity in DCT is only about 5% of that in skeletal muscle [27], and the PCT activity is lower still, by a factor of 100.

In any case, the fact that phosphocreatine is present at substantial levels in certain parts of the nephron, but is not an immediately available emergency source of high energy phosphate, suggests that it may have some other role in these regions. For example, phosphocreatine is known to be an inhibitor of both brain and muscle phosphofructokinase, a key control enzyme of the glycolytic pathway [28, 29].

Carbohydrate reserves. The only major energy reserves in kidney, besides nucleoside triphosphates and phosphocreatine, are glycogen and glucose. As seen, glycogen levels in the proximal tubule are almost negligible, whereas glucose levels are substantial—roughly equivalent to blood levels. In contrast, distal structures contain major concentrations of both glycogen and glucose. The glycogen levels approximate those of mouse brain [20], but are only about 10% of those of rat skeletal muscle [12] and 1% of those in liver [30]. However, the results show

that even where glycogen is relatively high it is not readily available during ischemia. For example, in the TAL after two minutes glucose was almost gone, whereas 65% of the glycogen remained. Glycogen phosphorylase data are not available for nephron segments, but Delaval et al [31] found activities of 200 and 300 mmol · kg^{-1} (dry wt) h^{-1} in rat kidney cortex and medulla, respectively. These values are 10% or less of the levels in fast twitch skeletal muscles, but are a third or more of levels in brain. Both of these are tissues which can mobilize their glycogen reserves rapidly during ischemia (brain) or with intense stimulation (muscle).

Thus lactate during brief ischemia comes chiefly from glucose, and as expected from regional studies and enzyme distribution data, the increases were much more rapid in distal than in proximal structures. The most relevant study is that of Bagnasco et al [4] who measured lactate production in isolated tubules with oxidation blocked by antimycin A. Highest activity was observed in (medullary) TAL, with almost zero production in proximal segments. The present results are in agreement, except that a significant increase in lactate was observed in the PST segments; the average for the first 45 seconds was 25% of that formed in TAL. The rate in TAL for the first 45 seconds was 27 mmol · kg^{-1} (dry wt) min^{-1} (27 pmol μg^{-1} · min^{-1}). On the basis of a dry weight for rat TAL of 0.188 $\mu g \cdot mm^{-1}$ (unpublished data of Dr. Helen B. Burch), this is a rate of 5.1 pmol · mm^{-1} · min^{-1}. This agrees surprisingly well with Bagnasco et al [4] who found an average of 5.4 pmol · mm^{-1} · min^{-1} in their study.

Glucose-6-phosphate decreased with ischemia in every region examined, except possibly the papilla. Hems and Brosnan observed a similar decrease in whole kidney, whereas in liver, they found that glucose-6-phosphate increased with ischemia [23]. They attributed the difference to the "absence in kidney of major glycogen stores." Actually, whether glucose-6-phosphate goes up or down with increased flux depends on the balance between activation of hexokinase and phosphorylase and availability of glycogen on one side, and activation of phosphofructokinase on the other. It is clear that in this case phosphofructokinase activation was sufficient in all parts of the nephron to more than compensate for an increase in glucose-6-phosphate formation. As noted, the time course for glucose-6-phosphate in TAL was exceptional in that after a very rapid fall during the first 5 or 10 seconds, there was almost no further change for the full two minutes, during which time lactate continued to accumulate. This probably indicates that phosphofructokinase was initially activated more rapidly than hexokinase (and phosphorylase), whereas after the first few seconds the activation of hexokinase was sufficient to maintain a relatively high, steady-state glucose-6-phosphate level. It is significant that the same phenomenon of a rapid fall in glucose-6-phosphate followed by a plateau was observed in mouse brain during ischemia [20].

This study may help explain why the distal tubule is more resistant to ischemia than the proximal [32]. After two minutes of ischemia both TAL and DCT had more residual ATP than the proximal segments and TAL, at least, had 75% of its relatively high glycogen reserve remaining. The proximal segments still had substantial glucose residues, but their capacity to use it is very poor.

Whether or not this speculation has validity, it does not help to explain why during anoxia with continuing filtration (in contrast to ischemia) the TAL is selectively damaged. Since this damage is prevented by blocking filtration or by giving inhibitors of Na transport [5], the first guess would be that the damage is due to continuing expenditure of ~P for Na transport in spite of the O_2 lack. A problem with this explanation is the paradoxical finding that addition to the perfusion fluid of inhibitors of the electron transport chain lessens TAL damage [33]. These additives lowered the perfusion rate, which should be protective, but they also lowered ATP levels in the *whole medulla*, which should be harmful, provided that the TAL shared in this general fall in ATP. However, this is not necessarily true. The TAL have a substantial glycogen reserve, as well as high levels of glycolytic enzymes, including hexokinase (note in Table 2 the high rate of lactate production.) This should make them less dependent on oxidative metabolism than the proximal tubules, and therefore able to survive with the extra aid from the decrease in perfusion. To settle this question, it should be useful to examine high energy phosphate compounds and metabolites in the TAL itself, during hypoxic perfusion experiments.

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Helen Burch died suddenly January 18, 1987 at the age of 80, shortly after this paper was accepted for publication. She had an unbroken productive research career spanning more than 50 years, and worked full time up to the day before she died. Dr. Burch was a pioneer in the study of renal biochemistry, and the changes which occur in specific nutritional diseases. She will be particularly remembered for her work on the quantitative histochemistry of the nephron.

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References

1. GUDER WG, ROSS BD: Enzyme distribution along the nephron. *Kidney Int* 26:101–111, 1984
2. BURCH HB, LOWRY OH, PERRY SG, FAN L, FAGIOLI S: Effect of age on pyruvate kinase and lactate dehydrogenase distribution in rat kidney. *Am J Physiol* 226:1227–1231, 1974
3. COHEN JJ, KAMM DE: Renal metabolism: Relation to renal function, in *The Kidney* (2nd ed), edited by BRENNER B, RECTOR FC. Philadelphia, Saunders, 1981, pp. 144–148
4. BAGNASCO S, GOOD D, BALABAN R, BURG M: Lactate production in isolated segments of the rat nephron. *Am J Physiol* 248: F522–F526, 1985
5. BREZIS M, ROSEN S, SILVA P, EPSTEIN FH: Renal ischemia: A new perspective. *Kidney Int* 26:375–383, 1984
6. VENKATACHALAM MA, BERNARD DB, DONOHOE JF, LEVINSKY NG: Ischemic damage and repair in the rat proximal tubule; differences among S_1, S_2 and S_3 segments. *Kidney Int* 14:31–49, 1978
7. OLIVER J: New directions in renal morphology: A method, its results and its future. *Harvey Lect* 40:102–155, 1944–1945
8. LOWRY OH, PASSONNEAU JV: *A Flexible System of Enzymatic Analysis*. New York, New York Academic Press, 1972, pp. 291
9. CHAN AWK, BURCH HG, ALVEY TR, LOWRY OH: A quantitative histochemical approach to renal transport. I. Aspartate and glutamate. *Am J Physiol* 229:1034–1044, 1975
10. KATO T, BERGER SJ, CARTER JA, LOWRY OH: An enzymatic cycling method for nicotinamide-adenine dinucleotide with malic

and alcohol dehydrogenases. *Anal Biochem* 53:86–97, 1973
11. CHI MM-Y, LOWRY CV, LOWRY OH: An improved enzymatic cycle for nicotinamide adenine dinucleotide phosphate. *Anal Biochem* 89:119–129, 1978
12. HINTZ CS, CHI MM-Y, FELL RD, IVY JL, KAISER KK, LOWRY CV, LOWRY OH: Metabolite changes in individual rat muscle fibers during stimulation. *Am J Physiol* 242:C218–C227, 1982
13. LOWRY OH, BERGER SJ, CHI MM-Y, CARTER JG, BLACKSHAW A, OUTLAW WH, JR: Diversity of metabolic patterns in human brain tumors. I. High energy phosphate compounds and basic composition. *J Neurochem* 29:959–977, 1977
14. LUST WD, PASSONNEAU JV, CRITES SK: The measurement of glycogen in tissues by amylo-α-1,4-α-1,6-glycosidase after the destruction of pre-existing glucose. *Anal Biochem* 68:328–331, 1975
15. LOWRY CV, KIMMEY JS, FELDER S, CHI MM-Y, KAISER KK, PASSONNEAU PN, KIRK KA, LOWRY OH: Enzyme patterns in single human muscle fibers. *J Biol Chem* 253:8269–8277, 1978
16. LIENHARD GE, SECEMSKI II: P^1, P^5-Di(adenosine-5′)pentaphosphate, a potent multisubstrate inhibitor of adenylate kinase. *J Biol Chem* 248:1121–1123, 1973
17. BURCH HB, CHOI S, DENCE CN, ALVEY TR, COLE BR, LOWRY OH: Metabolic effects of large fructose loads in different parts of the rat nephron. *J Biol Chem* 255:8239–8244, 1980
18. NEEDLEMAN P, PASSONNEAU JV, LOWRY OH: Distribution of glucose and related metabolites in rat kidney. *Am J Physiol* 215: 655–659, 1968
19. BURCH HB, HAYS AE, MCCREARY MD, COLE BR, CHI MM-Y, DENCE CN, LOWRY OH: Relationships in different parts of the nephron between enzymes of glycerol metabolism and the metabolite changes which result from large glycerol loads. *J Biol Chem* 257:3676–3679, 1982
20. LOWRY OH, PASSONNEAU JV, HASSELBERGER FX, SCHULZ DW: Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. *J Biol Chem* 239:18–30, 1964
21. GERLACH E, BADER W, SCHWOERER W: Uber den Stoffwechsel säurelöslicher Phosphor–Verbindungen in der Ratteniere. *Pflügers Arch* 272:407–433, 1961
22. NISHIITSUTSUJI–UWO JM, ROSS BD, KREBS HA: Metabolic activities of the isolated perfused rat kidney. *Biochem J* 103:853–862, 1967
23. HEMS DA, BROSNAN JT: Effects of ischemia on content of metabolites in rat liver and kidney *in vivo*. *Biochem J* 120:105–111, 1970
24. JONES NF, WELT LG: Adenosinetriphosphate in rat renal papilla: Effects of vasopressin and of ischemia. *Am J Physiol* 212:939–944, 1967
25. BALABAN RS, GADIAN DG, RADDA GK: Phosphorous nuclear magnetic resonance study of rat kidney in vivo. *Kidney Int* 20:575–579, 1981
26. RADDA GK, ACKERMAN JJH, BORA PJ, SEHR PA, ROSS BD, GREEN Y, BARTLETT S, LOWRY M: ^{31}PNMR studies on kidney intracellular pH in acute renal acidosis. *Int J Biochem* 12:277–281, 1980
27. HINTZ CS, LOWRY CV, KAISER KK, MCKEE D, LOWRY OH: Enzyme levels in individual rat muscle fibers. *Am J Physiol* 239:C58–C65, 1980
28. UYEDA K, RACKER E: Regulatory mechanisms in carbohydrate metabolism. VII hexokinase and phosphofructokinase. *J Biol Chem* 240:4682–4688, 1965
29. KRZANOWSKI J, MATSCHINSKY FM: Regulation of phosphofructokinase by phosphocreatine and phosphorylated glycolytic intermediates. *Biochem Biophys Res Com* 34:816–823, 1969
30. BURCH HB, LOWRY OH, BRADLEY ME, MAX JR PF: Hepatic metabolites and cofactors in riboflavin deficiency and calorie restriction. *Am J Physiol* 219:409–415, 1970
31. DELAVAL E, MOREAU E, ANDRIAMANANTSARA S, GELOSO J: Renal glycogen content and hormonal control of enzymes involved in renal glycogen metabolism. *Pediatr Res* 17:766–769, 1983
32. KAHNG MW, BEREZESKY IK, TRUMP BF: Metabolic and ultrastructural responses of rat kidney cortex to *in vitro* ischemia. *Exp Mol Pathol* 29:183–198, 1978
33. BREZIS M, SHANLEY P, SILVA P, SPOKES K, LEAR S, EPSTEIN FH, ROSEN S: Disparate mechanisms for hypoxic cell injury in different nephron segments. *J Clin Invest* 76:1796–1806, 1985